J. Agrobiotech. **Vol. 4**, 2013, p. 33–42.

ISSN 1985-5133 (Press)
ISSN 2180-1983 (Online)

Anatomical Study of Stem, Petiole, Leaf, Tuber, Root and Flower of *Dioscorea hispida* Dennst. (Dioscoreaceae) by Using Optical Microscope, SEM and TEM

Salmah Tajuddin[1], Nashriyah Mat[1], Abdul Ghani Yunus[1] and Shamsul Bahri A. R.[2]

[1]Faculty of Agriculture and Biotechnology, Universiti Sultan Zainal Abidin (UniSZA), Gong Badak Campus, 21300 Kuala Terengganu, Terengganu Darul Iman, MALAYSIA.
[2]Rubber Research Institute of Malaysia, P.O. Box 10150, 50908 Kuala Lumpur, MALAYSIA.

***Corresponding author; E-mail: salmahtajuddin@unisza.edu.my**

ABSTRACT

Observation under optical microscope, Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM) on stem, leaf, petiole, tuber, root and flower of *Dioscorea hispida* Dennst. presented detailed information of anatomical characters that defined this species. The anatomical study showed that *Dioscorea hispida* leaves had similar feature with eudicot plants, but the stem, tuber and flower resembled monocotyledonous plants. The leaf surface of *Dioscorea hispida* was covered with rough, bristly and spiny trichomes or hairy surface.

Keywords: *Dioscorea hispida*, optical microscope, Scanning Electron Microscope (SEM), Transmission Electron Microscope (TEM), anatomy

ABSTRAK

Kajian dengan menggunakan mikroskop optik, Mikroskop Elektron Pengimbas (SEM) dan Mikroskop Pancaran Elektron (TEM) telah dilaksanakan untuk mendapatkan maklumat terperinci ciri-ciri anatomi batang, daun, petiol, akar dan bunga *Dioscorea hispida* Dennst. yang menakrifkan spesies ini. Kajian anatomi menunjukkan daun *Dioscorea hispida* mempunyai sifat yang menyamai tumbuhan eudikot, manakala batang, ubi dan bunga menyerupai tumbuhan bermonokotiledon. Permukaan daun *Dioscorea hispida* adalah diselaputi oleh trikom kasar, berbulu dan berduri atau permukaan yang berbulu.

Kata kunci: *Dioscorea hispida*, mikroskop optik, Mikroskop Elektron Pengimbas (SEM), Mikroskop Pancaran Elektron (TEM), anatomi

INTRODUCTION

Dioscorea hispida Dennst. (intoxicating yam or *Ubi Gadung*) belongs to section *Lasiophyton*, within order Dioscoreales, in the Family Dioscoreaceae (Ayensu, 1972). This plant is a vine that climbs up other plants by twinning to the right. In particular, the leaves have a palmately compound leaf; veins are arranged in a net-like pattern that occurs in most eudicot. The tuber of *Dioscorea hispida* had been used as an exotic food as well as a famine food of importance in Malaysia, especially in Terengganu state, and some parts of the world. It is critically a neglected crop ever since rice became affordable as a food source. According to the Terengganu Forestry Department, the distribution of *Dioscorea hispida* in forest areas is very limited but it can be found in abundance near river. Land clearing and human habitation put significant pressure on *Dioscorea hispida*. Nevertheless, some local communities are still planting this species as a potential food resource and for medicinal purposes (Nashriyah *et al.,* 2012a).

The collection of both sexes of *Dioscorea hispida* had been difficult with limited knowledge and reference. *Flora Malesiana* described that male and female plants may be distinguished by capsular fruit. However, the capsular fruit only exists after the ovulation process. According to Edeoga *et al.* (1998), *Dioscorea hispida* is dioecious, whereby male and female flowers are borne on separate plants. Male flowers are small, insconspicuous and borne in panicles; while female flowers are relatively larger and borne on spikes. There is no other morphological parameter to distinguish between male and female plants.

Morphologically, the surface structure of *Dioscorea hispida* is covered by trichomes. The stem has a wide range in diameter, thick-walled, terete and stout, with thorny surface. The maximum growth period of stem is twelve months and the plant replaces the stem and tuber each year. The tuber is large, bristly; and globosely lobed that grows on an underground stem. Petiole length is up to 30 cm, with elongated petiolules; three foliolate leaves, with a size that can go up to 12-20 cm long. A leaflet is somewhat hairy, with palmate veins, a broad lamina with primary nerve reaching up to the apex. Leaf shape can be considered as a combination of ovate and elliptic with acuminate apex, entire margin and cordate base.

Dioscorea hispida is quite unique in a way that it produces a secondary growth of wood, and anatomical and taxonomic descriptions of *Dioscorea* spp. had been carried out extensively by researchers (Wilkin *et al.,* 2000; Wilkin *et al.,* 2002; Weber *et. al.,* 2005; Haigh *et al.,* 2005; Wilkin *et al.,* 2008; Wilkin *et al.,* 2009). The

objectives of this study were to identify and describe some anatomical features that define and delimit *Dioscorea hispida*. The histology, anatomy and ultrastructure of *D. hispida* were described in this study based on observations by conventional techniques using optical microscope, Transmission Electron Microscope (TEM) and Scanning Electron Microscope (SEM). TEM and SEM are the analytical instruments most widely used to reveal numerous structures, at a magnification above that obtainable in optical microscopes, in three dimensional configurations to support the taxonomy (Alberti and Nuzzaci, 1996).

MATERIALS AND METHODS

Sampling

In this study, *Dioscorea hispida* was identified by the local people and herbarium specimen collection. The live specimens were collected for the anatomical investigation in the laboratory. The plant samples were collected from the natural forest habitat on a riverside bushy hill, at the Perkampungan Orang Asli Jalan Genting Lama, 13 Miles off Jalan Gombak, Gombak, Selangor, Malaysia, and at Kampong Bari Besar, Setiu, Terengganu, Malaysia. Plant parts including stem, leaf, tuber and flowers that were used in this study were either fresh or fixed in fixative prior to dissection.

Optical Microscope

The critically small flower character was evaluated and observed through a dissecting microscope. A needle was used to gently open the flower for better infiltration in the fixation and embedding stage. The rest of the plant parts were directly cut using a sharp razor and fixed in formalin-acetic acid-alcohol (F.A.A) for optical view, in order to avoid any traumatic change and artifact contamination as much as possible. The samples were gradually passed through 100% TBA to remove and replace water with alcohol. Paraffin wax was then used to replace TBA for embedding. The embedded sample was sectioned between 5-10 micrometers thin, with a rotary microtome (AO-820 Spencer Rotary Microtome, American Optical), stained with safranin O and fast green, and permanently mounted on a slide for later viewing (Cutler *et al.,* 2008).

Scanning Electron Microscope (SEM)

A FEI Quanta 200 ESEM electron microscope and a JEOL JSM-6390 LA were used for observation at low vacuum capability between 10-130 Pa which enabled a charged-free imaging and analysis of non-conductive specimen. The instruments allowed direct examination of the wide-range surface of sample on the stab (Alberti & Nuzzaci, 1996).

Transmission Electron Microscope (TEM)

TEM observation was conducted for midrib and petiole parts of leaf only. The samples were fixed with 2.5% gluteraldehyde fixative in 0.1 M phosphate buffered saline (PBS) at pH 7.4 for two hours and under vacuum, to facilitate penetration (1^{o} fixation). Then, samples were rinsed repeatedly with the above buffer before and after secondary fixing through 2% osmium tetroxide, because osmium tetroxide would fix lipids well. To avoid any drastic environmental changes, specimens were dehydrated through a series of ascending percentage concentrations of ethanol solutions for 15 minutes. Propylene oxide series was replaced gradually and extended to resin infiltration. The vial was kept standing in a moving rotator to facilitate the infiltration. These samples were then polymerized in epoxy resin, in the oven at 70 ^{o}C for a day. The resulting polymerised resin blocks were sectioned at 60 – 100 nanometers thickness with an ultramicrotome (EM UC6, Leica). The sections were stained with uranyl acetate, and later with lead citrate. The sections were then viewed under a TEM (STEM CM12, Philips), and digital micrographs were captured and stored (Alberti & Nuzzaci, 1996).

RESULTS AND DISCUSSION

Stem

The aerial stem is an annual, right-twining vine, terete, stout, pubescent when young, glabrescent, with spines. Epidermis was composed of wax-coated dermal cells, thick-walled with undulating cuticle. Stem cells exhibited variable sizes and cuboidal starch grains. Epidermis, cortex, sclerenchyma and vascular bundle were clearly differentiated (Figure 1). Vascular bundles were found to be irregularly-scattered in the ground tissue constituting a V-shaped arrangement of metaxylem vessel and tracheids with large phloem units terminating the flanges of V (Ayensu, 1972). Each vascular bundle was covered with sclerenchyma cells.

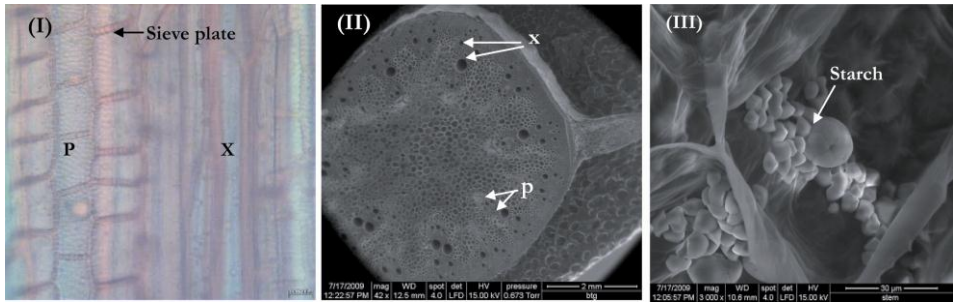

Fig. 1. Anatomy of *Dioscorea hispida* stem. (I) The stem was composed of xylem (x) and phloem (p) tissues as observed under optical microscope. (II) SEM micrograph of the general view of stem cross section (X42). (III) SEM micrograph of cuboidal starch grains in parenchyma cell (X3000) (x, xylem; p, phloem).

Petiole

The petiole had a similar structure to the stem with long, pentagonal, jointed basal pulvinus for each end, thin and soptically undulating cuticle, hairy surface and nine vascular bundles orientated in a ring around the marginal central cylinder. Each vascular bundle consisted of phloem, xylem and sclerenchyma (Figure 2). Crystal was present. TEM observation result indicated that cells in petiole were empty.

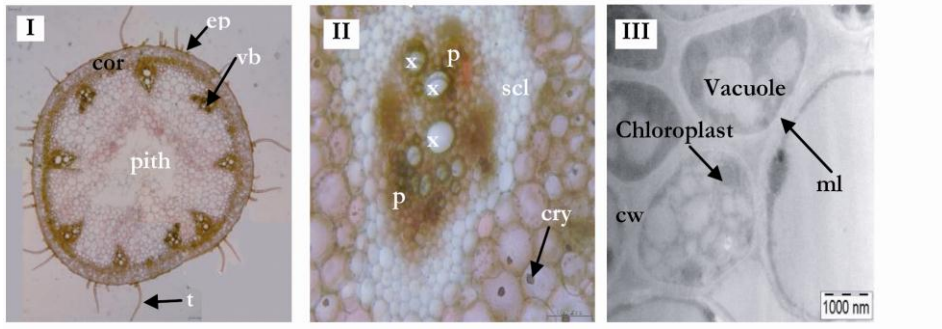

Fig. 2. Petiole anatomy of *Dioscorea hispida*. (I) General view of petiole with a thin and soptically undulating cuticle, a hairy surface and nine vascular bundles orientated in a ring around the marginal central cylinder. (II) Each vascular bundle consists of phloem, xylem and sclerenchyma. (III) TEM image of petiole shows vacuole, chloroplast and cell wall (t, trichome; ep, epidermis; vb, vascular bundle; scl, sclerenchyma.; x, xylem; p, phloem; cry, crystal; cw, cell wall; ml, middle lamella).

Leaf

The anatomical characteristics of *Dioscorea hispida* leaf featured a leaf covered with hairs, with jigsaw puzzle-shaped cells on a surface view (Figure 3). Only non-glandular hairs or trichomes were observed on the leaf midrib and lamina (Figure 3), but Nashriyah *et al.* (2012b) had observed both the glandular and non-glandular trichomes on the midrib and lamina of *D. hispida* leaves.

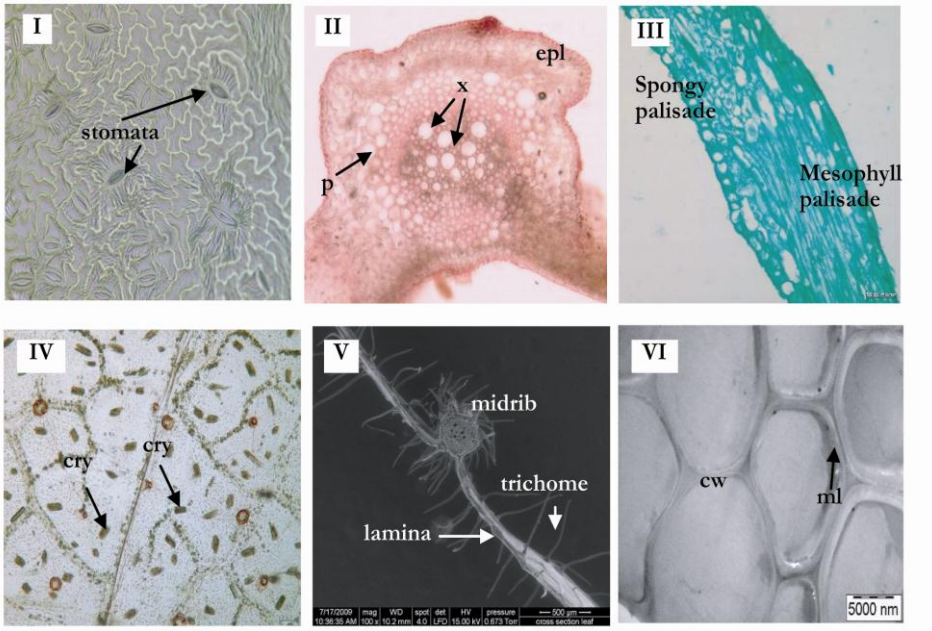

Fig. 3. Leaf anatomy, morphology and ultrastructures of *Dioscorea hispida*. (I) Abaxial view of cleared leaf, showing anomocytic stomata. (II) Leaf anatomy shows a cross section of midrib. (III) Longitudinal section of lamina. (IV) Raphide crystals present in leaf. (V) SEM micrograph of midrib shows the non-glandular trichomes that covered midrib and also the lamina (X100). (VI) TEM view of petiole shows thickened cell wall of cells and distinct middle lamella (epl, epidermis layer; x, xylem; p, phloem; cw, cell wall; ml, middle lamella; cry, crystal).

The lamina has 3-4 prominent veins extending from the base to the apex as in a typical eudicot plant. The lamina's cuticle was soptically thickened. The leaf has a layered epidermis, soptically sinuous wall on abaxial cells, while adaxial cells have a straight wall. Stomata could be found on the abaxial surface with irregular distribution and anomocytic type. Palisade tissue was clearly distinguished, which is elongated, compactly arranged and orientated in vertical, with transverse section on

adaxial side, while spongy tissue was either loosely or compactly arranged with irregular-size or -shaped cells. Raphide crystal development in mesophyll cells was traced (Figure 3).

Tuber

The tuber generally is renewed annually, occurring at the soil surface, large, lobed, covered with dead roots, bristly, and present numerous starch granules (Figure 4) in variable sizes and ovate-shape filling parenchyma cells. The cork layer was bright brown in color and occurred as a protective layer. These cork cells when dead became the bark at the outermost, as in a woody stem undergoing secondary growth, which was also shown in *Dioscorea alba*. In either white or yellow flesh tuber, it was protected by dioscorine that is a highly poisonous alkaloid which can paralyze the nervous system.

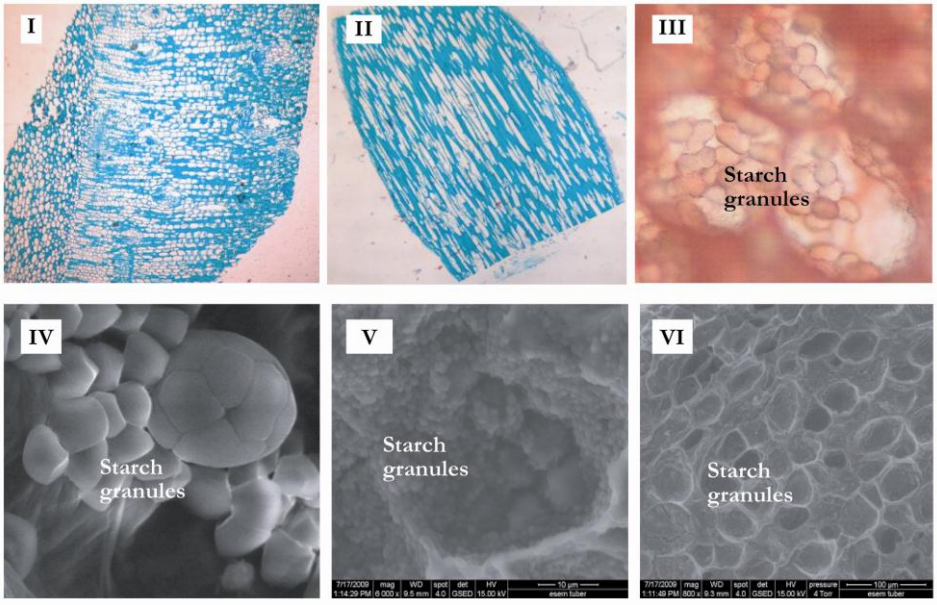

Fig. 4. Tuber anatomy and ultrastructures of *Dioscorea hispida*. (I) Cross section of tuber. (II) Longitudinal section of tuber. (III) Starch granules viewed under optical microscope. (IV) Cuboidal starch granules viewed under SEM. (V) SEM micrograph of starch granules (X6000). (VI) Starch granules were in abundance in the tuber cells (X800).

Root

Dioscorea hispida possess a fibrous root as in other monocotyledons which are short-lived, that form and die due to their fragile nature. The root develops from the tuber and also from the stem base. From our observation, this root was fully lignified. A thick layer of epidermis developed as a protective layer. The root contained a high number of xylem and phloem poles (Figure 5).

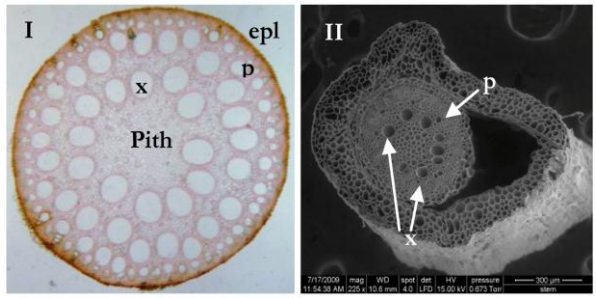

Fig. 5. Histology and morphology of *Dioscorea hispida* root. (I) Root cross section through light microscope showed vascular bundles scattered in a concentric ring. This vascular bundle arrangement is considered a eustele or separate vascular bundle in the cortex with phloem to the outside of the xylem. (II) SEM micrograph of a stele with pith in the middle (X225) (epl, epidermis layer; x, xylem; p, phloem).

Flower

Flowers are dioecious where male and female flowers are borne on different plants, thus plant collection for both sexes has been difficult. In this study, only male flowers were found. The flower is very small and massed together along flowering axes upward, with 40 flowers, alternate on rachis. Flowers are generally yellowish-green in color and covered with hair. The inner segment of the parianth is deltoid and the outer segment widely ovate, 6 stamens were borne at the base of the perianth, filaments terete thick, anthers oblong, bracteoles broadly ovate, and outer lobes smaller and thinner than inner lobes (Ridley, 1924). The stamens were clearly visible within the flower (Figure 6).

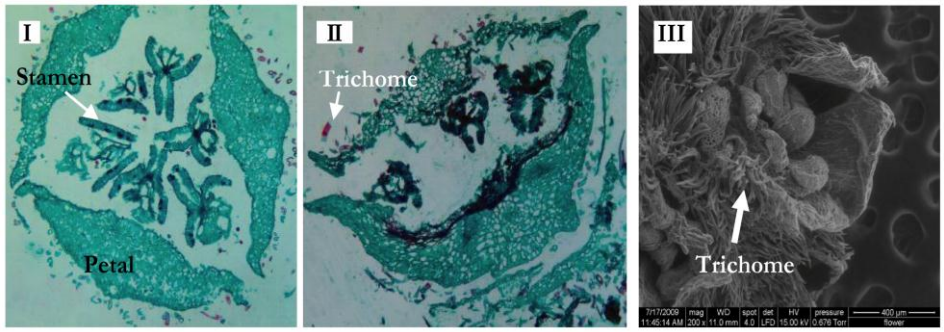

Fig. 6. *Dioscorea hispida* floral histology and morphology. (I) Cross section of a male flower under optical microscope. (II) Longitudinal section of a male flower under optical microscope. (III) A scanning electron micrograph clearly shows that the male flower was surrounded by hairs or trichomes (X200).

CONCLUSION

Information on anatomical characters of *Dioscorea* had been described by Ayensu (1972), thus already potraying a greater picture of the taxonomic status of this genus. *Dioscorea* characters were close to Smilacaceae family except for the inferior ovary, capsular fruit and large cavity albumen (Ayensu, 1972). The results from this study would contribute to the taxonomy of *D. hispida* species from its anatomical pattern. This study has revealed that *Dioscorea hispida* leaves had similar features of a eudicot plant, but stem, tuber, seed and flower resembled the monocotyledonous plant. The present observations indicated that *Dioscorea hispida* surface area was covered with trichomes, giving it a rough, bristly or hairy surface. The vascular bundle structure was composed of V-shaped terminating arrangement of metaxylem vessel, tracheids and phloem units. Starch granule distribution was observed in the stem, petiole, petiolules and tuber. Raphide crystal was present in the leaf mesophyll tissue.

ACKNOWLEDGEMENTS

This work was carried out under a UniSZA and RRIM collaborative research project. Laboratory facilities were provided by RRIM and SEM facilities were provided by Jeol (Malaysia) Sdn. Bhd. and Fei Worldwide Corporation.

REFERENCES

Alberti, G. & Nuzzaci, G. 1996. 1.6.5 SEM and TEM techniques. *World Crop Pests* **6**: 399-410.

Ayensu, E. S. 1972. *Anatomy of the Monocotyledons: VI Dioscoreales.* Oxford University Press. 182 pp.

Cutler, D. F., Botha, T. & Stevenson, D. M. 2008. *Plant Anatomy: An Applied Approach.* Blackwell Publishing Ltd. 302 pp.

Edeoga, H. O., Okoli, B. E. & Agba, I. 1998. Observations on the floral morphology of some *Dioscorea* species (Dioscoreaceae) in Nigeria. *Folia Geobotanica* **33:** 201-205.

Haigh, A., Wilkin, P. & Rakotonasolo, F. 2005. A New Species of *Dioscorea* L. (Dioscoreaceae) from Western Madagascar and its distribution and conservation status. *Kew Bull.* **60:** 273-281.

Nashriyah M., Salmah T., Nur Atiqah M. Y., Siti Nor Indah O., Muhamad Azhar A. W., Munirah, S., Nornasuha Y. & Abdul Manaf A. 2012a. Ethnobotany and distribution of *Dioscorea hispida* Dennst. (Dioscoreaceae) in Besut, Marang and Setiu Districts of Terengganu, Peninsular Malaysia. *World Academy of Science, Engineering and Technology* **72:** 240-243.

Nashriyah M., Salmah T., Nornasuha Y., Norhayati N., Nurrul Akmar R., Khairil M., Syahril A. H., Abdul Ghani Y. & Shamsul B. 2012b. Trichomes on *Dioscorea hispida* Dennst. leaves. p. 459-462. In: *Taxonomy and Ecology: Beyond Classical Approaches.* B. A. Fasihuddin, M. Sepiah, I. B. Ipor, R. Zainudin, M. E. Wasli, K. Meekiong & Z. Assim (eds.). Universiti Malaysia Sarawak, Kota Samarahan, Sarawak, Malaysia. 488 pp.

Ridley, H. N. 1924. *The Flora of the Malay Peninsula Vol. IV (Monocotyledones).* L. Reeve & Co. Ltd., London, U.K. 383 pp.

Weber, O., Wilkin, P. & Rakotonasolo, F. 2005. A new species of edible yam (*Dioscorea* L.) from Western Madagascar. *Kew Bull.* **60:** 283-291.

Wilkin, P., Caddic, L., Foster, C. & Schols, P. 2000. A new species of *Dioscorea* L. (Dioscoreaceae) from Eastern Madagascar and its pollen morphology. *Kew Bull.* **55:** 427-434.

Wilkin, P., Rakotonasolo, F., Schols, P. & Furness, C. A. 2002. A new species of *Dioscorea* L. (Dioscoreaceae) from Western Madagascar and its pollen morphology. *Kew Bull.* **57:** 901-909.

Wilkin, P., Andrianantenaina, W. P., Jeannoda, V. & Hladik, A. 2008. The species of *Dioscorea* L. (Dioscoreaceae) from Madagascar with campanulate tori, including a new species from Eastern Madagascar. *Kew Bull.* **63(4):** 583-600.

Wilkin, P., Hladik, A., Weber, O., Hladik, C. M. & Jeannoda, V. 2009. *Dioscorea orangeana* (Dioscoreaceae), a new and threatened species of edible yam from Northern Madagascar. *Kew Bull.* **64:** 461-468.